Nonprotein Amino Acids in the Murchison Meteorite

KEITH A. KVENVOLDEN, JAMES G. LAWLESS, AND CYRIL PONNAMPERUMA

Exobiology Division, Ames Research Center, NASA, Moffett Field, California 94305

ABSTRACT **Twelve nonprotein amino acids appear to be present in the Murchison meteorite. The identity of eight of them has been conclusively established as *N*-methylglycine, β-alanine, 2-methylalanine, α-amino-*n*-butyric acid, β-amino-*n*-butyric acid, γ-amino-*n*-butyric acid, isovaline, and pipecolic acid. Tentative evidence is presented for the presence of *N*-methylalanine, *N*-ethylglycine, β-aminoisobutyric acid, and norvaline. These amino acids appear to be extraterrestrial in origin and may provide new evidence for the hypothesis of chemical evolution.**

The formation of organic compounds and their accumulation have been considered to be a necessary preamble to the appearance of life on the primordial earth (1). Various forces, such as ultraviolet light from the sun, heat from volcanos, electrical discharges in the form of lightning, and ionizing radiation from radionuclides acting upon the reducing atmosphere of the primitive earth may have produced a large number of organic compounds until the early oceans had the consistency of a "hot dilute soup" (2–5). Considerations of stellar and planetary evolution lead us to believe that the sequence of events that led to life on earth may have been duplicated elsewhere in innumerable planetary systems in the universe (6–9).

In attempting to substantiate this hypothesis, two avenues have generally been used. In the synthetic approach, the conditions of a primitive planet have been simulated in the laboratory. Many of the constituents of proteins and nucleic acids have been synthesized in this manner (10, 11). In analytical studies, investigations have centered around the interstellar medium, lunar samples, ancient sediments, and meteorites. Radioastronomical observations have clearly demonstrated the presence of the smaller molecules important to chemical evolution, such as water, ammonia, hydrogen cyanide, formaldehyde, cyanogen, carbon monoxide, and cyanoacetylene, as components of interstellar matter (12). The analysis, however, of lunar samples from the Apollo 11 and 12 missions (13–15) showed no conclusive evidence of any molecules of significance to chemical evolution. The oldest sediments available on the earth, dated at about 3.1–3.4 billion years, are reported to contain microscopic evidence of early life (16, 17). On account of the apparent absence of unmetamorphosed samples older than these rocks, and because any prebiotic terrestrial organic matter would probably have been consumed by microorganisms once life arose, the possibility of discovering any molecules predating the emergence of life appears to be remote.

The analysis of meteorites has provided some evidence for the presence of extraterrestrial carbon compounds (18). Carbonaceous chondrites, for example, are known to contain up to 5% carbon (19, 20). The finding of amino acids indigenous to such samples would constitute convincing evidence for extraterrestrial chemical evolution. In our analysis of the Murchison meteorite we have already reported (21) the presence of five amino acids commonly found in protein: glycine, alanine, valine, proline, and glutamic acid, and two not generally found in proteins: *N*-methylglycine (sarcosine) and α-aminoisobutyric acid (2-methylalanine). We have now identified aspartic acid and six additional nonprotein amino acids: β-alanine, α-amino-*n*-butyric acid, β-amino-*n*-butyric acid, γ-amino-*n*-butyric acid, isovaline, and pipecolic acid. Furthermore, there appears to be tentative evidence for at least four other nonprotein amino acids: β-aminoisobutyric acid, norvaline, *N*-methylalanine, and *N*-ethylglycine.

ANALYSIS

The amino acids were obtained from the meteorite by extraction of a pulverized sample with boiling distilled water, followed by acid hydrolysis of the aqueous extract (22). After the removal of inorganic salts from the acid hydrolysate, the resulting material was analyzed for amino acids by ion-exchange chromatography, gas chromatography, and gas chromatography combined with mass spectrometry. The residue left after the water extraction of the meteorite was hydrolyzed with acid and similarly analyzed. In a procedural blank, no amino acids could be detected.

A piece of the Murchison meteorite (23) that appeared to be massive in character, and showed signs of the least exterior contamination, was treated in the following manner. The ablation crust was first chiselled away. The interior pieces were then pulverized for 15 sec in an Angstrom Disc Mill (T100).

10 g of the pulverized meteorite were refluxed with 50 ml of triply-distilled water for 20 hr at about 110°C. The extract was recovered, after centrifugation, by decantation. The residue was twice rinsed with 50 ml of water. The extract and rinses were evaporated to dryness, and hydrolyzed with 50 ml of 6 N HCl for 20 hr at about 110°C. The resulting hydrolysate was again evaporated to dryness.

The dried hydrolysate was dissolved in about 10 ml of water and charged on a 50-ml column packed with Dowex 50 (Bio-Rad) ion-exchange resin, prepared in the H⁺ form. The column was successively eluted with two bed volumes of water and two bed volumes of 2 N NH₄OH. The NH₄OH eluate was collected, evaporated to dryness, and redissolved in 5 ml of water; 0.5 ml was removed for analysis by ion-exchange chromatography using a conventional amino acid analyzer. In a subsequent experiment on a different sample, the procedure included use of Dowex 50 (H⁺) followed by Dowex 1 (OH⁻).

Ion-exchange chromatography

The pH of the 0.5-ml solution was adjusted to about 3 and 1 ml of pH 2.2 buffer was added. This mixture was charged on the column of an automatic amino acid analyzer (Spinco Model 120) for determination of neutral and acidic amino acids.

Preparation of diastereoisomeric derivatives for gas chromatography

The remaining 4.5 ml of solution were evaporated to dryness. *N*-trifluoroacetyl-D-2-butyl esters of amino acids were prepared (24). The dried residue from the aqueous solution was refluxed for 3 hr at 110°C under anhydrous conditions with 0.5 ml of D-2-butanol–8 N HCl (Kroon Research) which was 96% enriched in the D isomer. Excess D-2-butanol was removed by evaporation. To the product was added 0.5 ml of dichloromethane and 0.05 ml of trifluoroacetic anhydride (Eastman). The mixture reacted at room temperature for 16 hr and was evaporated at room temperature. Dichloromethane was added and portions of this solution were analyzed by gas chromatography and gas chromatography–mass spectrometry. Diastereoisomeric derivatives of amino acid standards were prepared by using DL-2-butanol instead of D-2-butanol.

Gas chromatography

Gas chromatographic analyses were performed on Perkin Elmer 881 and 900 gas chromatographs (flame-ionization detection) on two different capillary columns (150 ft × 0.02 in.); one was coated with UCON 75 H 90,000 and the other with XE 60 (both phases available through Applied Science).

Gas chromatography–mass spectrometry

The effluent from the Perkin Elmer 881 gas chromatograph (with the UCON 75 H 90,000 column installed) was introduced into a CEC 21-491 mass spectrometer through a membrane separator and the mass spectra of the eluted compounds were obtained.

Hydrolysis of meteorite residue

The meteorite residue, after water extraction, was hydrolyzed with 50 ml of 1 HCl under reflux for 20 hr at about 110°C. This hydrolysate was treated in a manner similar to that described for the hydrolysate of the water extract.

IDENTIFICATION

Ion-exchange chromatography with an automated amino acid analyzer revealed the presence of several common amino acids in the hydrolyzed extract as well as in the hydrolysate of the residue. The concentration of compounds in the latter was about four times smaller than in the hydrolyzed aqueous extract. In this paper we have, therefore, directed our attention to the amino acids in the hydrolyzed aqueous extract.

We have previously reported (21) that the hydrolyzed aqueous extract of this meteorite contained the amino acids glycine (6 μg/g), alanine (3 μg/g), valine (2 μg/g), proline (1 μg/g), and glutamic acid (3 μg/g). We had also found the nonprotein amino acids *N*-methylglycine and α-aminoisobutyric acid. The identity of these compounds was established by gas chromatography and gas chromatography combined with mass spectrometry. It was further shown that the enantiomers of each of the amino acids with asymmetric centers, namely alanine, valine, proline, and glutamic acid, were present in about equal amounts.

By a detailed examination of the gas chromatograms and mass spectra of this meteorite extract, we have found ten additional nonprotein amino acids. The identities of six of these have been definitely established. The identification of the four remaining may be considered tentative. Of the amino acids found in protein, we have also identified D- and L-aspartic acid, and confirmed the presence of L-glutamic acid, the mass spectrum of which we had not obtained previously (21).

The identification of the amino acids was based on the following criteria.

(*1*) Equivalence of the mass spectrum of the isolated amino acid with that of an authentic standard. The compounds compared were *N*-trifluoroacetyl-2-butyl esters of amino acids.

(*2*) Coincidence of retention times resulting from the cochromatography of the *N*-trifluoroacetyl-2-butyl ester of the isolated amino acid with the derivative of the corresponding standard.

Table 1 lists the nonprotein amino acids whose presence was established by these criteria. Fig. 1 illustrates the gas-chromatographic separation of the derivatives of these compounds. In four cases where the spectra obtained from the sample resembled the standard, but were weak, the identifications were considered tentative. Cochromatography of standards supported the tentative identifications.

The most abundant ion fragments for the nonprotein amino acids identified in this meteorite sample are shown in Table 2. Mass spectra of the *N*-trifluoroacetyl-2-butyl esters of authentic amino acid standards resemble closely, with a few exceptions, the mass spectra of *N*-trifluoroacetyl-*n*-butyl

TABLE 1. *Nonprotein amino acids in the Murchison meteorite*

Chromatographic peak number (Fig. 1)	Name	Supplier of authentic amino acid standard†
10	*N*-methylglycine (sarcosine)*	K & K
20	β-alanine	K & K
5	*N*-methylalanine (DL?) (tentative identification)	Cyclo
11	*N*-ethylglycine (tentative identification)	Cyclo
2	α-aminoisobutyric acid (2-methylalanine)*	Eastman
6 and 8	α-amino-*n*-butyric acid (D and L)	K & K
14 and 15	β-aminoisobutyric acid (D and L) (tentative identification)	Calbiochem
16	β-amino-*n*-butyric acid (DL?)	Mann
23	γ-amino-*n*-butyric acid	Mann
1	Isovaline (DL?)	K & K
12 and 13	Norvaline (D and L) (tentative identification)	Mann
17 and 18	Pipecolic acid (D and L)	Calbiochem

* Previously identified (21).

† The identities of the standards were supported by mass spectra obtained for *N*-trifluoroacetyl-*n*-butyl and *N*-trifluoroacetyl-DL-2-butyl ester derivatives.

TABLE 2.   *Most abundant ion fragments of the nonprotein amino acids in the Murchison meteorite*

m/e	N-methyl-glycine	β-alanine	α-aminoiso-butyric acid	α-amino-n-butyric acid	β-amino-n-butyric acid	γ-amino-n-butyric acid	Isovaline	Pipecolic acid
100							6	
101							3	
102			3	4				
110	2							
112	5				7	8		
114			10		9	4	8	
116		7			2	3	2	
126	4	28		9		26	3	6
127		6				7		
130				2	8			
139		12			4	9		
140	100	5		15	80	12	1	
141	43	5			7			
153			4	5	18	7		
154			100	100	4	28		
155			16	46	9	4		
166	4	3	2		7		10	
167	1	4					6	
168	27	100					100	
169	2	7					12	
180			2	4	5	2		100
181					5	14		10
182			12	16	100	100		1
184							12	
185	15	8						
186	2	10						
194							2	
195							1	
196							6	
199					11	15		
200					18	5		
208								7
225								4
240							1	
241	2							
281								5

esters (Lawless and Chadha, manuscript in preparation). The mass spectra taken during the gas-chromatographic separation suggest the presence of at least two other isomers of valine (M^+ 269), and two other homologs of proline (M^+ 281) (isomers of pipecolic acid). A major problem, however, in the identification of these compounds is the unavailability of standards, without which identification is equivocal.

Three nonprotein amino acids, isovaline, β-amino-n-butyric acid, and N-methylalanine, all of which have asymmetric carbon atoms, did not yield two peaks, corresponding to each diastereoisomeric derivative, when the N-trifluoroacetyl-DL-butyl esters of these amino acid standards were chromatographed both on UCON 75 H 90,000 and on XE 60. For this reason, it was impossible to estimate the ratio of D and L isomers for these compounds as found in this meteorite. Consequently, the expression (DL?) is used in Table 1 in the case of these amino acids.

CONCLUSION

The analytical procedures of ion-exchange chromatography, gas chromatography, and gas chromatography combined with mass spectrometry have established unequivocally that six

amino acids commonly found in protein, and eight which do not occur in natural protein, are present in the Murchison meteorite. All the amino acids that have asymmetric carbon atoms and whose diastereoisomeric derivatives could be separated by the gas chromatographic method we used appeared to consist of approximately equal amounts of the D and L isomers.

The possibility of terrestrial contamination is inherent in meteorite analysis. Recent biological contamination would, however, invariably lead to the predominance of L isomers of the amino acids commonly found in protein (25, 26). The identification of nonprotein amino acids with an almost equal abundance of D and L isomers argues strongly against this possibility.

If the amino acids are indigenous to the meteorite and therefore extraterrestrial, the question immediately arises as to their origin. Two possibilities present themselves: these amino acids were present at some period of time in the meteorite in one stereoisomeric form and were then racemized in the course of time (27, 28), or the two forms were always present in nearly equal amounts. The possibility that an extraterrestrial biota was responsible for D or L amino acids initially cannot be dis-
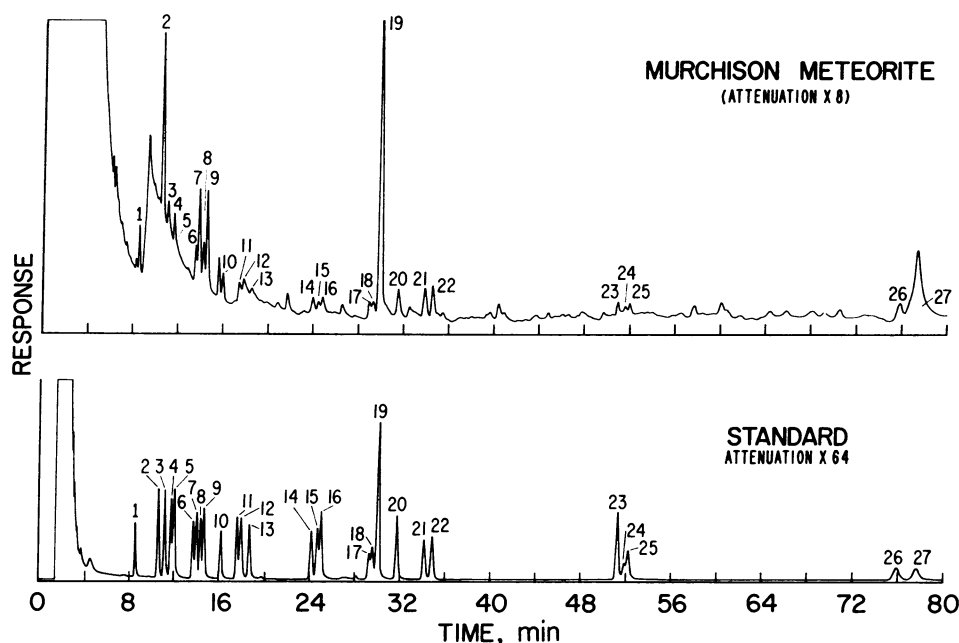
FIG. 1. Gas chromatogram of N-trifluoroacetyl-D-2-butyl esters of amino acids in acid-hydrolyzed aqueous extract of Murchison meteorite compared with a gas chromatogram of N-trifluoroacetyl-DL-2-butyl esters of standard amino acids. Gas chromatography: 0.02 inch $\times$ 150 foot capillary column coated with UCON 75 H 90,000; temperature programmed from 100 to 150°C at 1°C/min. Identification for the sample is as follows: (1) (DL?)-isovaline; (2) α-aminoisobutyric acid; (3) D-valine; (4) L-valine; (5) (DL?)-N-methylalanine (?); (6) D-α-amino-n-butyric acid; (7) D-α-alanine; (8) L-α-amino-n-butyric acid; (9) L-α-alanine; (10) N-methylglycine; (11) N-ethylglycine (?); (12) D-norvaline (?); (13) L-norvaline (?); (14) D-β-aminoisobutyric acid (?); (15) L-β-aminoisobutyric acid (?); (16) (DL?)-β-amino-n-butyric acid; (17) D-pipecolic acid; (18) L-pipecolic acid; (19) glycine; (20) β-alanine; (21) D-proline; (22) L-proline; (23) γ-amino-n-butyric acid; (24) D-aspartic acid; (25) L-aspartic acid; (26) D-glutamic acid; (27) L-glutamic acid (masked by, an unidentified peak in Murchison analysis). The same identifications hold for the chromatogram of the standard, except for the compounds represented by peaks (1), (5), and (16). These compounds are known to be DL.

counted entirely. However, by analogy with terrestrial life, it would be difficult to explain the presence of several nonprotein amino acids. The production of either the D or the L form by an abiogenic process and subsequent racemization may also be considered unlikely, since no well-defined evidence is available for abiotic processes that would produce one form rather than the other, in spite of extensive experimentation (29). We are therefore led to believe that the amino acids in the Murchison meteorite were most likely produced in both forms (DL) by an abiotic process. Of the twelve nonprotein amino acids positively and tentatively identified by us, five have been identified in laboratory experiments simulating primitive earth or planetary conditions (30, 31). The presence of all the isomers of the amino acids of two and three carbon atoms and all but two of the isomers of the amino acids with four carbon atoms, suggests a random synthesis. In addition, the previously reported random distribution of isomers of aliphatic hydrocarbons and heavy (^{13}C) isotopic values of the extractable carbon (21), point to an abiogenic origin of the organic matter in this meteorite. We conclude that our analysis of the Murchison meteorite provides a new basis for the study of chemical evolution and the search for extraterrestrial life (9).

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